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Cell Implantation

Principal Investigator: Bakay, Roy A
Grant Number: 1R01NS046612-01A1
Title: Stem Cells in CNS Transplantation

Abstract: Stem cells offer tremendous promise for the future of transplantation. We propose examining embryonic stem cells (ESC) in monkey allografts. We will compare dopaminergic enriched ESC to fetal mesencephalic (FM) neurons in their ability to survive, innervate, and restore lost function in the best animal model of PD, the MPTP treated monkey. The primate is essential for this study to test the hypothesis that replacement strategy must completely reinnervate the very large volume of the monkey striatum. Recently clinical trials have indicated that dopaminergic (DAergic) replacement with FM neurons can cause severe debilitating dyskinesia. It is then imperative to have a clear understanding of how a DAergic enriched ESC replacement strategy affects I-dopa-induced dyskinesia (LID). In this regard, we will also compare the effects of FM transplants and DAergic enriched ESC upon the dyskinesia profile of MPTP monkeys. The potential to induce or diminish dyskinesia will be tested with the best model of dyskinesia (primate LID model). The key problem of parkinsonian transplantation with fetal or stem cells grafts is the incomplete reinnervation of host striatum. Like the FM transplant patients, focal areas of relative hyperdopaminergic activity should render these monkeys highly susceptible to LIDs. Thus to optimize reinnervation and functional recovery while minimizing the potential for dyskinesia, we will also treat DAergic enriched ESC with glial cell line-derived neurotrophic factor (GDNF) delivered via a lentiviral vector. The lenti-viral vector is critical to this hypothesis because of the proven ability to transfect the entire striatum and act not as a point source but as a volume source to stimulate reinnervation. Intraparenchymal GDNF released diffusely throughout the entire striatum should act as a developmental cue for these immature cells to extend DAergic processes throughout the striatum as well as provide neuronal rescue for dopaminergic neurons in the pars compacta of the substantia nigra. Sufficient subjects and multiple controls are included to insure proper interpretation of the data. The present series of experiments serves to provide the essential preclinical data needed to help determine the utility of nonhuman dopaminergic enriched stem cells. -

Principal Investigator: Bohn, Martha C
Grant Number: 5R01NS031957-08
Title: GENE THERAPHY FOR PARKINSON'S DISEASE

Abstract: The long-term goal of this project is to develop novel gene therapies for neurodegenerative diseases. In the previous support period, we focused on adenoviral (Ad) vectors to deliver the gene encoding GDNF (glial cell line-derived neurotrophic factor). Ad-GDNF injected into either the substantia nigra or striatum of a progressive degeneration model of Parkinson's disease protected dopaminergic (DA) neurons against cell death induced by the neurotoxin 6-OHDA. Ad-GDNF injected into the striatum also prevented the acquisition of behaviors and molecular changes that occurred in DA deficient young and aged rats. This proposal focuses on the hypothesis that anti-apoptotic gene delivery will also protect DA neurons in vitro and in vivo and have a synergistic effect with delivery of neurotrophic factor genes. Viral vectors harboring genes that block specific apoptotic death pathways, including XIAP, a dominant-negative caspase-9, bcl-2 and bclxl will be studied for effects on survival and function of DA neurons either alone or in combination with neurotrophic factors, GDNF or neurturin. Genes will be delivered to DA neurons in culture and in rat brain using helper free HSV:AAV hybrid amplicon vectors. These vectors will incorporate bidirectional expression cassettes that drive both the therapeutic gene and the cellular marker gene, green fluorescent protein, to permit specific evaluation of transduced cells. Expression will be controlled using the tetracycline responsive element such that transgene expression is "on" in the presence of tetracycline activator (TA) and in the absence of doxcycline (Dox). Vectors will be made in which TA is driven by a viral promoter of the DA cellular promoter, tyrosine hydroxylase (TH). Effects of the 'therapeutic' genes will be studied using non-neuronal cells, the DA cell line, MN9D, and primary fetal DA neurons treated with the neurotoxins, MPP+ or 6-OHDA or other cellular insults. In vivo effects of therapeutic genes will be studied in: 1) rats that have received grafts of fetal DA neurons, and 2) rats that have received a progressive 6-OHDA lesion of the nigrostriatal projection. Reversibility of effects will be studied by administration of Dox. Effects on DA neurons will be evaluated using quantitative morphometric and molecular techniques and behavioral evaluations. This project also aims to continue its evaluation of new generation viral vectors, including E2b deleted Ad, totally gutted Ad, and HSV:AAV amplicon, for stability and levels of expression in the nigrostriatal system. The studies involve collaborations among investigators at Children's Memorial Hospital and Northwestern Univ. Med. School and are relevant to the development of novel therapies for neurodegenerative diseases and injuries to the CNS. -

Principal Investigator: BULTE, JEFF W
Grant Number: 5R01NS045062-02
Title: MR Tracking of Magnetically Labeled Stem Cells in CNS

Abstract: Unavailable

Principal Investigator: COLLIER, TIMOTHY J.
Grant Number: 5R01NS042125-04
Title: Cell Grafts for Parkinson's Disease

Abstract: In vitro expansion of neural progenitor cells followed by induction of dopaminergic phenotype may provide a limitless source of cells for grafting into patients with Parkinson's disease (PD). However, the signals controlling the conversion of these cells into dopamine (DA) neurons must be identified. In an effort to accomplish this, single cells isolated from ventral mesencephalon were clonally expanded and exposed to hematopoeitic cytokines and neurotrophic molecules. Analysis of cell differentiation in response to this treatment yielded conversion of a high percentage (72 to 98 percent) of cells in some clones to a tyrosine hydroxylase (TH)-positive phenotype. Of the 24 clones generated, the best conversion to TH cells occurred with exposure to a combination of interleukin-1 (IL-1), interleukin- 11 (IL-11), leukemia inhibitory factor (LIF), and glial cell line-derived neurotrophic factor (GDNF). Positive clones expressed TH, the DA transporter, Nurr-1 and released DA in culture. Other cells in cytokine-exposed clones expressed GFAP (astrocyte marker) or MAP-2 (neuron marker) indicating that the original neurospheres were also capable of producing clones that differentiate into glial and nondopaminergic neurons. Initial neural grafting studies m the rat model of PD using a clone with the highest conversion rate to TH indicated that converted progenitor cell grafts produced complete amelioration of amphetamine-induced rotational behavior and continued to express the TH phenotype. However, the survival rate of these grafted progenitor cells was reduced (26 percent) compared to embryonic ventral mesencephalon (VM). The experiments proposed here will develop protocols for optimal survival of Wafted cytokine-converted mesencephalic progenitor cells. Once survival of grafted mesencephalic progenitor cells is optimized, direct comparisons will be made to fresh embryonic VM grafts on measures of behavior, in vivo dialysis, post-mortem DA biochemistry, DA receptors, cell survival and neurite extension. Lastly, this proposal will test the efficacy of the DA conversion cocktail on clonal progenitors derived from embryonic mesencephalon of nonhuman primate brain. If successful, cytokine-converted mesencephalic progenitor cells could potentially replace embryonic tissue as the primary source of cells for grafting in PD. -

Principal Investigator: HEIDENREICH, KIM A
Grant Number: 5R01NS038619-05
Title: IMPROVING SURVIVAL OF TRANSPLANTED DOPAMINE NEURONS

Abstract: Fetal dopamine neurons are implanted into the brains of Parkinson's disease patients in an effort to replace lost neurons and restore dopamine levels. Death of 95% of the transplanted neurons limits the usefulness of this experimental therapy. Transplanted dopamine neurons die in part by apoptosis (programmed cell death) and to a lesser extent by necrosis. This finding opens the exciting possibility that signaling pathways leading to the initiation and execution of the death program can be blocked while signaling pathways that protect against apoptosis can be activated to reduce neuronal death. In vitro experiments have shown that p38 mitogen-activated protein (MAP) kinase is activated during apoptosis in primary neurons and that a specific inhibitor of p38 MAP kinase rescues neurons from apoptotic cell death. Since apoptosis occurs in neural grafts during transplantation, we hypothesize that specific inhibitors of p38 MAP kinase will block apoptosis of fetal dopaminergic neurons transplanted into the striatum of Parkinsonian rats resulting in increased neuronal survival, better reinnervation of the striatum, and improved motor behavior. IGF-1, bFGF, insulin and GDNF also rescue cultured dopaminergic neurons from apoptosis. Based on studies in other types of neurons, these growth factors are thought to work by either stimulating antiapoptotic pathways, (i.e., IGF-1 stimulation of Akt) or inhibiting proapoptotic pathways (i.e., insulin inhibition of p38 MAP kinase). Since there are multiple pathways regulating apoptosis, we predict that combination of growth factors and inhibitors of p38 MAP kinase will provide additive protection of the transplanted grafts against apoptosis. Moreover, by further delineating the signaling pathways that mediate the antiapoptotic effects of the above growth factors, new cellular targets for therapy can be defined. The specific aims of the proposal are to: #1. Determine if specific inhibitors of p38 MAP kinase improve transplantation of fetal dopaminergic neurons. #2. Determine if the combination of p38 MAP kinase inhibitor and growth factors provides additive protection against apoptosis in transplanted tissue grafts. #3. Define the signaling pathways by which IGF-1, bFGF, insulin and GDNF promote survival of dopaminergic cells. Results from these studies will provide a basis for translating the use of p38 inhibitors and growth factors in transplantation to human studies and will provide new cellular targets that can be manipulated to prevent or arrest neuronal apoptosis. -

Principal Investigator: IACOVITTI, LORRAINE M
Grant Number: 3R21NS043705-02S1
Title: Neural Stem Cells Grafts in Primate Models of Parkinsons

Abstract: Unavailable

Principal Investigator: IACOVITTI, LORRAINE M
Grant Number: 2R01NS032519-11A1
Title: Studies of Purified Dopamine Neurons

Abstract: Historically, there has been no good way to isolate DA neurons from other cells of the midbrain. Thus, missing DA neurons have been replaced by mixed cell populations following transplantation of embryonic midbrain tissue in animal models of disease and in Parkinson's patients. Although, in many cases, these transplants have provided long-term benefit, the presence of unwanted cells, such as glia, non-DAergic neurons, or even excessive numbers of DA neurons, has produced serious side effects, and in rare cases, even death. Discovering ways in which to segregate DA neurons from other cell types poses a significant challenge, but a necessary next step. In the present proposal, our plan is to take advantage of several new advances in the laboratory; including the recent cloning of 11kb human tyrosine hydroxylase gene promoter (hTH). This sequence accurately targets the expression of the reporter, green fluorescent protein (GFP) to DA neurons of the mammalian CNS. Because GFP can be directly visualized in live fetal DA neurons, this approach allows enrichment via flourescent activated cell sorting (FACS) for study in vivo and in vitro. Moreover, it is possible to adapt these purification methods to mouse stem and human progenitor cells using a lentiviral vector to transduce cells with the hTH-GFP transgene. Following their DA differentiation and FACS sorting, our goal is to study purified populations of engineered stem/progenitor-derived DA neurons in culture or after transplantation into the Parkinsonian rat. These models offer us a unique opportunity to determine the ideal number of DA neurons needed as well as the optimal conditions which contribute to their survival and growth following transplantation. Graft function will be assessed in live animals via behavioral testing and in vivo microdialysis which will be correlated with biochemical and anatomical (at the light and electron microscopic levels) changes following sacrifice. This work will hopefully lay the foundation for the development of therapeutic treatments for Parkinson's and other diseases involving compromised DA systems.
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Principal Investigator: IACOVITTI, LORRAINE M
Grant Number: 5R01NS043309-03
Title: Using Stem Cells in Animal Models of Parkinson's Disease

Abstract: One promising new therapy for Parkinson's Disease (PD) involves the replacement of degenerated nigrostriatal neurons with those derived from transplanted fetal mesencephalic tissue. Although this approach has often yielded remarkable recovery of function in rats and monkeys, results in clinical trials with PD patients have been less consistent. At issue, is the relative inability to standardize a number of critical factors in human fetal transplants, including the age, type, number and integrity of cells being grafted. Consequently, finding more reliable sources of dopaminergic (DA) tissue for transplantation has become increasingly important. One direction has been to search for a line of readily available, well-characterized continually self-renewing stem or precursor cells that possess the capacity to differentiate, ideally spontaneously and with the need for little manipulation, into DA neurons, thus providing an inexhaustible and uniform source of replacement tissue. Towards this end, our preliminary findings demonstrate that grafts of embryonic mouse neural stem cells (NSCs) of the C17.2 cell can differentiate exclusively into neurons, which in a majority of cases, can express DA traits when cells are transplanted into the brain of a Parkinsonian rat. In addition, in preliminary studies using stem cells from adult human bone marrow (MSCs), we have found that nearly 100 percent of MSCs will convert into process- bearing, beta-tubulin III+ neuronal-like cells after only 1-2 hours of incubation with specific differentiation factors. If these cells also exhibit the same capacity as NSCs to respond to appropriate DA differentiation cues in vivo, patients could provide their own source of stem cells for autologous grafts in PD. Using NSC and MSC stem cell models and a multidisciplinary approach, our specific goals for this proposal are threefold: 1) Identify the conditions that promote the stable appearance of a postmitotic differentiated DA phenotype in stem cells grown in culture; 2) Identify those factors which promote the differentiation of a DA phenotype in transplanted stem cells and 3) Determine whether the DA phenotype in transplanted stem cells is stable and long lasting, and whether, it can produce functional recovery of motor deficits in a rat model of PD. The ultimate goal of this research program is a fuller understanding of the cellular and molecular processes regulating the differentiation of DA traits in stem cells and apply that knowledge to transplantation strategies for the treatment of Parkinson's Disease.-

Principal Investigator: **ISACSON, OLE**
Grant Number: **3P50NS039793-05S1**
Title: **NOVEL THERAPEUTIC APPROACHES FOR PARKINSON'S DISEASE**

Abstract: Unavailable

Principal Investigator: **KORDOWER, JEFFREY H**
Grant Number: **5R01NS043290-03**
Title: **DYSKINESIAS IN LENTI-GDNF TREATED PARKINSONIAN MONKEYS**

Abstract: Fetal nigral grafts can cause "runaway" dyskinesias in patients with Parkinson's disease (PD;Freed et al., 2001). These dyskinesias are severe, debilitating and strongly indicate that 1) novel dopaminergic surgical therapeutic strategy planned for clinical trials need to be tested preclinically for their effects upon dyskinesias and 2) the mechanisms underlying these dyskinesias need to be elucidated. We have recently demonstrated that lentiviral gene delivery of glial cell-derived neurotrophic factor (GDNF) potently prevents motor dysfunction and prevents nigrostriatal degeneration in nonhuman primate models of PD (Kordower et al., 2000). Prior to initiating clinical trials with lenti-GDNF, it effects upon dyskinesias need to be evaluated in parkinsonian monkeys. Freed, Fahn and coworkers (2001) have hypothesized that grafted-mediated dyskinesias result from graft overgrowth. However, their own PET and post-mortem data, as well as the data from others (Kordower et al., 1995, Lee et al 1999), do not support this view. We propose an alternative hypothesis that these dyskinesias result from local "hot spots" of hyperdopaminergic function interacting with the levodopa primed brain. We plan to test this hypothesis by comparing gene therapies that induce either a) widespread or b) local hyperdopaminergic function upon dopa-induced dyskinesias and the role of dopa priming. This application will have three Specific Aims. Specific Aim 1 will test the hypothesis that lenti-GDNF treatment to non-levodopa primed MPTP-treated monkeys will prevent, or diminish the intensity of dyskinesias when they are later treated with levodopa. Specific Aim 2 will test the hypothesis that lenti-GDNF will diminish the dyskinesia profile in dyskinesic MPTP-treated monkeys previously primed with levodopa. Specific Aim 3 will test the hypothesis that "hot- spot" hyperdopaminergic function, but not homogenous hyperdopaminergic innervation, will enhance the dyskinesia profile of parkinsonian monkeys and that elimination of GDNF will reverse the functional and dyskinesic effects established previously by this trophic factor. The study of dyskinesias has become a compelling area of PD research. Exciting therapeutic strategies such as gene therapy need to be evaluated for their effects on dyskinesias so that they are both safe and effective. This application will determine whether potent dopaminergic gene therapies influence dyskinesias in the best animal model of PD.-

Principal Investigator: LI, JIA-YI
Grant Number: 5R21NS043717-02
Title: ADULT STEM CELL THERAPY IN PARKINSON'S DISEASE

Abstract: Objective: The aim of this project is to develop a novel source of adult stem cells as an alternative to embryonic-derived stem cells/tissue for neural grafting in Parkinson's disease (PD). Bone marrow-derived hematopoietic stem cells and brain-derived adult ependymal stem cells will be investigated with respect to their potential to differentiate into dopaminergic (DA-ergic) neurons in vitro: In a final phase, the cells will be grafted into PD animal models. The work program includes (phase 1) isolation and purification of both cell types by using specific markers and magnetic sorting or FACS. In phase 2, cells will be propagated in vitro and characterized; respective mitogenes screened and protocols optimized. Phase 3 involves the identification of factors promoting neuronal/DA-ergic differentiation for the respective cell types and optimization of differentiation protocols in vitro. Cells will be characterized morphologically by immunocytochemistry and functionally by measuring K+-stimulated DA release in vitro. Subsequently, to verify a possible clinical application of the investigated cell types for neural grafting, undifferentiated as well as differentiated cells will be transplanted intracerebrally in rat/mouse models of PD in phase 4. Grafted cells will be assessed morphologically by immunohistochemistry, their ability to form synaptic contacts with the host brain by staining for synaptic vesicle proteins (such as synaptophysin) in combination with confocal and electron microscopy. Functionality of grafted cells will be tested by rotational behavior pre- and post transplantation. The project is aimed at further understanding of neural stem cell biology and more importantly, to use a highly goal-derived approach to develop a translation protocol for adult-derived stem cells that can be readily applied in future clinical trials in PD. -

Principal Investigator: LI, SENLIN
Grant Number: 1R01NS046004-01A1
Title: Macrophage Gene Therapy of Neurodegenerative Diseases

Abstract: Neurodegenerative diseases affect a large population of patients. Existing therapies are not satisfactory. Gene therapy holds promise, but focal delivery of DNA and the level of gene expression are challenging. Macrophages are recruited from bone marrow to most tissues of the body including the CNS, thus making them an attractive option for gene delivery. Galactosialidosis (GS) has been corrected by bone marrow-derived macrophages expressing human protective protein/cathepsin A (PPCA) transgene in a mouse model (PPCA-/-). However, correction in the CNS was incomplete due in part to weakness of the CSF-1R promoter used in the study. We have developed a series of super macrophage promoters (SMP) that are up to l00-fold stronger in vitro than the CSF-1R promoter. In models of the highly prevalent Parkinson's disease (PD), local delivery of glial cell line-derived neurotrophic factor (GDNF) has been found beneficial. We hypothesize that highly effective CNS delivery of GDNF can be achieved with the use of our super macrophage promoters and this will greatly ameliorate the pathologic changes and neurological defects in animal models of PD. To explore this hypothesis, our specific aims are: 1) To characterize these super macrophage promoters by transplantation of bone marrow stem cells transduced ex vivo with lentiviral vectors and in transgenic mice using EGFP (enhanced green fluorescent protein) as a reporter. Promoters with the greatest strength and tissue-specificity for macrophages will be used in the subsequent aims. 2) To ameliorate neurodegeneration in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease by syngeneic transplantation of HSC transduced ex vivo with lentivectors expressing GDNF gene in macrophages/macroglia driven by the SMP. Bone marrow stem cells will be transduced ex vivo with GDNF expressing lentivirus and transplanted into lethally irradiated recipient mice. Four weeks after bone marrow transplantation, the recipient mice will be injected subcutaneously with MPTP. At selected time points post MPTP administration, PET scan and behavioral testing will be performed, and brain tissue will be examined for dopamine uptake and expression of tyrosine hydroxylase (TH). In the substantia nigra pars compacta (SN), dopaminergic neurons will be counted and cell apoptosis will be assessed by TUNEL staining and immunohistochemistry for active easpase-3. 3) To ameliorate neurodegeneration in the same way as in Aim 2, but GDNF expression will be controlled by a tetracycline-regulatable gene expression system. To evaluate the effects of macrophage/ super promoter-mediated delivery and expression of GDNF on degenerating

Principal Investigator: Mckay, Ronald
Grant Number: 5Z01NS002981-06
Title: Stem Cell Biology And Brain Disease

Abstract: Unavailable

Principal Investigator: Mckay, Ronald
Grant Number: 5Z01NS002881-12
Title: The Molecular Biology Of The Mammalian Brain

Abstract: Unavailable

Principal Investigator: MOSLEY, RODNEY L
Grant Number: 1R21NS049264-01
Title: Neuroprotective Vaccination for Parkinson's Disease

Abstract: Microglia inflammation contributes, in significant measure, to the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) during idiopathic Parkinson's disease (PD). Attenuation of such inflammation could attenuate disease. To this end we show that microglial deactivation responses, induced by vaccination, in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) intoxicated mice improves dopaminergic neuronal survival. This was achieved by adoptively transferring spleen cells from copolymer-1 (Cop-1) immunized mice to MPTP-treated recipients. Spleen cells from ovalbumin (OVA) injected mice failed to affect neuronal protection. Thus, our preliminary works show that protection from dopaminergic neurodegeneration can be achieved by adaptive immunity with T cells specific for Cop-1. Based on response kinetics, antigen specificity, and functional adaptive T cell immune responses, we predict that the mechanism(s) of neuroprotective immunity can be realized and could provide novel treatment strategies for human disease. Our hypothesis posits that protection from dopaminergic neurodegeneration by Cop-1 vaccination is generated through immune cell-mediated mechanisms with specificity for Cop-1 peptides and self-antigens. To investigate this we will adoptively transfer T lymphocytes, B cells and monocytes from Cop-1 immunized mice into MPTP-treated animals. Neuroprotection will be assessed by numbers of dopaminergic neurons, neurotransmitter levels, and neuronal metabolites by magnetic resonance spectroscopic imaging (MRSI). Immune cell populations, proven relevant to neuroprotection will be evaluated for the expression of gene products that are cell population specific as candidates for neuroprotection. Genetic fingerprint analysis will include cDNA microarray analysis and proteomics. This approach takes advantage of an integrated and well-established research program within the Center for Neurovirology and Neurodegenerative Disorders and builds upon research activities in PD supported previously through private donations. These approaches could prove useful for treatment of human PD. -

Principal Investigator: REDMOND, D EUGENE
Grant Number: 5P01NS044281-02
Title: Improving neural graft function in parkinsonian monkeys.

Abstract: The benefits of fetal neural transplantation in primate Parkinson's models have been partially confirmed by studies in patients, but transplantation may have significant problems which should be addressed. Functional improvement appears variable, less effective in older patients, and incomplete in spite of some apparent increases in dopamine production. The hypotheses are that transplantation's limitations result from inadequate grafts, due to poor survival of implanted cells, lack of critical growth factors, or nonphysiological graft placements and distribution. This program proposes to test these hypotheses with strategies which may improve functional benefits--the primary outcome measure of all studies in MPTP parkinsonian monkeys. Project One targets early cell death after grafting, with strategies to reduce oxidant stress, hypoxia/ischemia, and apoptosis using cell adhesion factors, the lazaroid tirilizad mesylate, melatonin, vascular endothelial growth factor, and cAMP. Project Two focuses on growth factors produced by fetal striatum enriched in astrocyte progenitor cells, or the growth factor, GDNF, delivered from encapsulated cells. An optimized method will be tested to determine benefits of combined methods in young adult and aged monkeys. Project Three aims to restore the relevant dopamine pathways by implantation of substantia nigra (SN) precursor tissue into SN and directing its outgrowth to the target areas, using co-grafted fetal striatal cells, or GDNF delivery. Duration of and stability of behavioral improvement, possible dyskinesias, or other toxic effects will be evaluated for three years and compared with striatal grafts. Quantitative behavioral effects will be correlated with biochemical and morphological measurements post-mortem. These studies may contribute to improving graft survival, reinnervation, and physiological restoration of the defective dopamine circuits and normalizing function. Although considerable preliminary work has been done in rodents, and because definitive controlled experiments with verifiable outcomes cannot be accomplished in humans, hypotheses and safety should be tested in the MPTP model in monkeys. The projects will be undertaken jointly by the program investigators, applying the resources of a unique primate transplantation laboratory (Core A) and shared outcome methodologies, all coordinated by a program support unit (Core B). Understanding of fetal precursor cell survival and outgrowth may also lead to improved understanding of the plasticity and function of other potential replacement cells, such as stem cells, and be relevant to other human neurodegenerative or traumatic conditions in addition to Parkinson's disease.-

Principal Investigator: REDMOND, D EUGENE
Grant Number: 5R01NS040822-03
Title: Human Neural Stem Cells in Primate Parkinson's Model

Abstract: This project will study the hypothesis that human neural stem cells (hNSCs) implanted into monkeys can normalize parkinsonism resulting from the neurotoxin 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP). These primordial, uncommitted, pluripotent cells can be propagated in large numbers and then safely differentiated into most cell types of the nervous system, including dopamine-producing neurons. NSCs migrate to populate developing or degenerating brain regions, perhaps allowing a more functionally correct and effective reconstruction. Pilot studies now show engraftment of hNSCs in the brain of fetal, neonatal, infant, and adult monkeys, for at least a month. Dopamine depleted adult monkeys showed graft-derived tyrosine hydroxylase positive cells and appropriate migration from the site of injection to dopamine-depleted areas. This project will test hypotheses in monkeys: (1) that hNSCs will survive, differentiate, and integrate in the brain of normal adult monkeys without immunological rejection or harmful overgrowth; (2) that hNSCs will eliminate parkinsonism after MPTP treatment, and that the presence of dopamine injury will influence their distribution and fate. NSCs will be identified and quantitated using genetic markers, immunohistochemistry, and multi-synaptic tract tracing. The following will be characterized and compared in normal monkeys and monkeys after MPTP: hNSC survival, migration, cell division, differentiation, connectivity, immunogenicity, stability of expression of a transgene (LacZ), apoptosis, and effect of host environment on all of these. In the dopamine-depleted parkinsonian monkey, dopamine and its metabolite concentrations, autoradiography of dopamine transporters, behavioral reversal of parkinsonism, dose effects, and synaptic connections will be studied over time courses of 7 days, 1, 3, 6, and 12 months. Comparisons will also be made with effects of primary fetal ventral mesencephalic tissue transplants in parkinsonian monkeys from prior and parallel studies. These studies will advance our understanding of the neurobiology and safety of human neural stem cells in a well established clinically relevant primate model of Parkinson's disease, and, if successful, support safe clinical studies in patients with Parkinson's disease in the future. The results will also advance understanding of useful methods for studying and treating a broad range of neurodegenerative, genetic, and traumatic conditions of the nervous system. -

Principal Investigator: REUBINOFF, BENJAMIN
Grant Number: 5R01NS046559-02
Title: Functional dopamine neurons from ES cells

Abstract: Parkinson's disease is a common neurodegenerative disorder that results from degeneration of dopamine (DA) neurons in the nigro-striatal system. Transplantation of fetal DA neurons can relieve Parkinsonism in some patients; however, limited tissue supply is a major obstacle for widespread use of fetal cells. Human embryonic stem (hES) cells could provide the platform for creating an unlimited supply of human DA neurons for cell therapy of Parkinson's disease. The goal of this study is to develop DA neurons from hES cells (NIH registration code ES01-06) and to demonstrate their function and therapeutic potential in animal models of Parkinson's disease. We have recently developed highly-enriched (>95 percent) cultures of expandable, developmentally competent neural progenitors (NPs) from hES cells. The NPs differentiate spontaneously into neurons expressing tyrosine hydroxylase (TH), however, at a low frequency. Our preliminary data suggest that defined signals can significantly promote the differentiation of hES cell-derived NPs towards TH+ neurons. In this study we will further develop the protocols to direct the differentiation of hES cells into TH+ neurons by the following approaches: (A) Administration of growth factors and cytokines that are known to induce a midbrain fate. (B) Forced expression of key transcription factors in the development of DA neurons. (C) Co-culture with stromal cells that have DA fate-inducing activity. Potential synergism between the strategies will be determined. We will evaluate whether hES cell-derived TH+ neurons have electrophysiological and functional properties expected from midbrain DA neurons and whether they can lead to recovery in the rat model of Parkinson's disease. Our preliminary results suggest that transplantation of hES cell-derived NPs to the DA-depleted striatum of rats results in behavioral recovery of DA-mediated motor asymmetry. Lastly, we will evaluate the potential of hES cell transplantation to correct behavioral deficits and the abnormal electrical activity of basal ganglia neurons in the MPTP primate model, which most reliably mimics the human disorder. This study will pave the way for further developments that may eventually allow the use of human ES cells as an unlimited source of midbrain neurons for transplantation in Parkinson's disease.-

Principal Investigator: RICHARDSON, ROBERT M
Grant Number: 5F31NS042420-04
Title: Telomerase re-expression in postmorterm CNS Progenitors.

Abstract: Transplantation strategies using CNS stem cells offer tremendous potential for replacing neuronal circuitry lost to Parkinson?s disease and other neurological disorders. The ability of CNS grafts to survive after transplant is crucial if therapeutic benefit is to be provided to a large number of patients with PD. Additionally, there must be an adequate source of donor cells. The purpose of this proposal is to insert the human telomerase gene (hTERT) into human postmortem-derived neural progenitor cells (HPCPs) in order to improve the survival of transplanted cells and ultimately increase the proliferation of desired cell types. HPCPs provide an easily accessible donor source, and ectopic expression of hTERT is a logical means by which to immortalize these cells in culture and decrease their susceptibility to apoptotic death following transplantation. Determining the effect of ectopic telomerase expression in HPCPs on population doublings, resistance to apoptosis and ability to differentiate into all CNS cell types is the crucial first step in investigating these methods. Assessing the viability, proliferation and differential fates of hTERT+ HPCPs transplanted to a 6-OHDA lesioned rat model of Parkinson?s disease, and evaluating functional recovery in transplant recipients will further characterize the extent to which these approaches may contribute to future stem cell therapy for neurological disorders. For the treatment of Parkinson? disease, a future step may include combining these methods with strategies likely to induce a dopaminergic fate among a subset of these progenitors.-

Principal Investigator: SORTWELL, CARYL E
Grant Number: 1R03NS048188-01
Title: Evaluation of Hypoxia in Grafted Dopamine Neurons

Abstract: Intracerebral neural grafting strategies for neurological disorders are limited by the poor survival rate of grafted cells. For example, the survival rate of dopamine (DA) neurons grafted in parkinsonian animal models and in clinical trials with Parkinson s patients is merely 5-20%. Critical to clinical success is the development of methods whereby grafted DA neuron viability and reinnervation of the host striatum are markedly increased. The focus of this R03 application is to directly examine the role of hypoxia in intracerebral grafts utilizing a well-established paradigm, grafts of mesencephalic DA neurons. Our research indicates that massive apoptosis of grafted mesencephalic cells occurs within the first few days after transplantation and then sharply diminishes. This time course of grafted DA neuron death closely parallels the delay in host vascularization of the grafted cells. The lack of blood-borne oxygen, or hypoxia, experienced by the grafted cells during the immediate post-grafting interval is a likely candidate to trigger apoptotic cell death. However, the role of hypoxia in limiting graft survival has never been directly assessed. The overall hypothesis of this proposal is that grafted DA neuron survival is severely limited by hypoxia during the early post-transplantation interval when grafted cells are not adequately vascularized. Identification of hypoxia as a significant constraint on graft survival would direct future strategies aimed at enhancing intracerebral grafts of numerous cell types (primary cells, stem cells) implanted to treat a wide range of neurological disorders.-

Principal Investigator: STEECE-COLLIER, KATHY
Grant Number: 5R01NS045132-02
Title: LEVODOPA DYSKINESIAS: IMPACT OF DOPAMINE NEURONS

Abstract: Recent findings from long-term clinical grafting trials for Parkinson's disease (PD) show that a portion of graft recipients develop aggravated post-graft dyskinesias. These dyskinesias are severe, debilitating and strongly indicate that mechanisms underlying them need to be elucidated. Freed, Fahn and coworkers have hypothesized that grafted-mediated dyskinesias result from graft overgrowth. However, their own PET and post-mortem data, as well as the data from others, do not support this view. We propose an alternative hypothesis that post-graft worsening of dyskinesias result from local "hot spots" of hyperdopaminergic function interacting with the levodopa primed brain. We plan to test this hypothesis by comparing neural grafting strategies that induce either a) widespread or b) local hyperdopaminergic function upon dopa-induced dyskinesias AND the role of dopa priming in a rat model of parkinsonism. We, and others have demonstrated that unilaterally dopamine (DA) depleted rats chronically treated with levodopa exhibit dyskinesias with characteristics remarkably similar to the dyskinesias seen in human PD. Further, this animal model importantly displays basal ganglia mechanisms that allow for DA grafts to either accentuate (Steece-Collier et al, submitted) or ameliorate these dyskinesia indices, similar to that seen in human graft recipients. Prior to continued clinical use, a systematic evaluation of the interaction of neural grafting with levodopa dyskinesias is needed to ensure that this experimental therapy is both safe and effective. This rodent model provides a valuable first step in such a systematic evaluation of levodopa/graft interactions. These studies will provide important guidelines useful in developing primates studies where further hypotheses and verification can be tested. -

Principal Investigator: SUBRAMANIAN,
Grant Number: 7R01NS042402-04
Title: Intranigral Transplantation in Parkinsonian Monkeys

Abstract: Recent investigations indicate that dopaminergic (DArgic) neurons in the substantia nigra (SN) secrete dopamine not only in their axonal terminals within the striatum but also via their dendrites within the SN pars reticulata (SNr) and that loss of dopamine in the SNr may have a role in the development of parkinsonism in primates. As a corollary, restoration of both nigral and striatal dopamine inputs may produce better recovery of function in Parkinson's disease than restoration of dopamine inputs in the striatum alone. Therefore, the PI proposes to examine the effects of combined DArgic fetal ventral mesencephalic (FVM) cell transplantation into the SN and the striatum in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-treated hemiparkinsonian (HP) monkeys and compare the results with FVM transplants in the striatum or SN alone. Animals will be periodically assessed by investigators blinded to the type of transplantation using a behavioral battery of tests (BBT). All animals will be treated with intracarotid MPTP injections to cause a stable HP state and briefly treated with oral levodopa to verify responsiveness to DArgic therapy prior to randomization into 4 equal groups (1-4). Microelectrode recordings of neuronal activity and magnetic resonance imaging (MRI) will be used to guide all transplantation procedures. In specific aim 1 (SA 1), group 1 animals will receive simultaneous FVM transplants into both striatum and the SN, group 2 animals will receive striatal FVM transplants, group 3 animals will receive FVM transplants into the SN and group 4 animals will receive "control" fetal tissue transplants into the SN. Periodic BBT assessments and immunochemical assessment of the transplanted animals compared across groups 1-4 will be used to test the hypothesis that combined striatal and nigral FVM transplants ameliorates parkinsonism to a greater extent than striatal FVM or nigral FVM transplants alone. In SA 2, neuronal recordings will be obtained before and after tissue transplantation from all 4 groups of animals from the SNr and the subthalamic nucleus (STN) and compared. This experiment will examine the hypothesis that striatal FVM transplantation will alter neuronal discharge patterns in both SNr and in the STN, while nigral FVM transplantation will alter neuronal discharge patterns in the SNr only. In SA 3, dopamine levels will be measured in vivo using microdialysis before and after nigral FVM transplantation from the SN and STN in group 3 and group 4 animals. This experiment will test the hypothesis that nigral FVM transplants restore dopamine content in the SN but do not effect dopamine content in the STN. These 3 experiments will objectively evaluate the role of restoring DArgic

Principal Investigator: **YUREK, DAVID M**
Grant Number: **5R01NS042862-03**
Title: **Gene Therapy, Neural Grafts & Parkinson's Disease**

Abstract: Clinical trials have provided encouraging evidence that grafts of fetal dopamine neurons are an effective therapeutic approach toward counteracting the symptoms of Parkinson's disease. Modest therapeutic benefits are observed in grafted patients despite clinical and experimental evidence that survival of grafted cells is low and graft reinnervation is incomplete. The poor survival and limited fiber outgrowth may be a consequence of neural grafts placed ectopically into an environment where the grafted neurons do not receive the proper signals for successful growth and integration into the neural circuitry of the host brain. Gene therapy may be a viable technique to introduce factors [neurotrophic factors] into brain tissue that can potentiate the survival and functional outgrowth of neural grafts, and thus improve the therapeutic value of the graft. In the proposed studies, regulated viral vectors will be injected into the lesioned nigrostriatal pathway of rodents with experimental Parkinson's disease in order to induce transgene expression of several neurotrophic factors that have a history of providing potent neurotrophic support for dopamine neurons. Subsequently, neural grafts will be implanted into lesioned/transduced brain sites and the survival, reinnervation, and function of the grafts will be assessed. Because Parkinson's disease has a higher incidence in the elderly than in the younger population, and recent experimental evidence suggests that the expression of endogenous neurotrophic factors are diminished in the aged striatum following a neurodegenerative lesion, experiments will be performed in young, middle-age, or old rats with experimental Parkinson's disease and the results will be compared within and between each age group. The studies are designed to determine the optimal temporal expression of neurotrophic factors [GDNF, BDNF, FGF-2] that improve graft development and function using regulated viral neurotrophic factors [GDNF, BDNF, FGF-2] that improve graft development and function using regulated viral vectors in young and aged animals with experimental Parkinsonism. These studies will also determine if combinations of viral vectors expressing different neurotrophic factors can be used to improve the therapeutic effects of dopamine grafts.-

Principal Investigator: **ZHANG, SU-CHUN**
Grant Number: **5U01NS046587-02**
Title: **Stem Cell Therapy For Parkinson's Disease**

Abstract: Parkinson's disease (PD) results from the progressive loss of dopamine (DA) neurons in the midbrain. Replacement of the lost DA neurons with fetal midbrain cells through neural transplantation in clinical trials has produced clinical benefits and has laid a foundation for cell therapy in PD. This therapy, however, is hindered by the limited supply of effective donor cells. Human embryonic stem (hES) cells (NIH Registry WA01 and WA09), established from the inner cell mass of a preimplantation embryo, are capable of almost unlimited proliferation in an undifferentiated state, yet retain the potential to differentiate into almost all cell and tissue types of the body including DA neurons. Thus ES cells may provide a simple and continual source of specialized human cells, which can be standardized and banked. This application is to resolve a single but crucial issue surrounding potential stem cell replacement therapy for PD, i.e., which hES-derived cell type, neuroepithelial cells, DA neuron progenitors, or DA neurons, is best for transplant therapy in PD. This study is based on our success in guiding hES cells to neuroepithelial cells, DA neuron progenitors and mature DA neurons in culture. The criteria for determining the candidate cell type include safety to recipients, efficacy of the cells for functional replacement, efficiency in cell production, and simplicity for standardization of cell preparation procedures. The proposed study will determine the ideal hES-derived cells for PD therapy, thus leading to preclinical studies to transplant the selected cells into a monkey PD model we have established, and to bank and/or standardize the cell production in our Biomanufacturing Facility before clinical trials.-

Principal Investigator: Ziemba, Kristine S
Grant Number: 1F30NS048716-01
Title: Reconstruction of the nigrostriatal pathway

Abstract: The long-term objective of this research proposal is to develop a means to reconstruct the neural circuit that degenerates in Parkinson's disease (PD) - that is, the nigrostriatal pathway. While current therapy (levadopa treatment) for PD may alleviate symptoms for a while, there is still no way to halt or reverse the neurodegeneration. Since 1% of the population over the age of 65 is affected by PD, and the prevalence increases with increasing age, research into better therapies and an eventual cure for PD is important for our aging population. Cellular replacement is not a new idea in PD research, but this proposal differs from most previous efforts by attempting an anatomically and physiologically correct reestablishment of the nigrostriatal pathway, effecting a more complete behavioral recovery. Molecular cues to guide growth of dopaminergic neurons will be identified in vitro, and adenoviral vectors will be used to express these molecules between the substantia nigra (SN) and the striatum in hemiparkinsonian rats. When dopaminergic neurons are subsequently transplanted into the SN, their axons should grow along the growth-supportive pathway, ending in the striatal target. Success will be evaluated with detailed histological and behavioral analyses.-